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## Air and Surface Sampling Method for Assessing Exposures to Quaternary Ammonium Compounds Using Liquid Chromatography Tandem Mass Spectrometry

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### Abstract

This method was designed for sampling select quaternary ammonium (quat) compounds in air or on surfaces followed by analysis using ultraperformance liquid chromatography tandem mass spectrometry. Target quats were benzethonium chloride, didecyldimethylammonium bromide, benzyldimethyldodecylammonium chloride, benzyldimethyltetradecylammonium chloride, and benzyldimethylhexadecylammonium chloride. For air sampling, polytetrafluoroethylene (PTFE) filters are recommended for 15-min to 24-hour sampling. For surface sampling, Pro-wipe® 880 (PW) media was chosen. Samples were extracted in 60:40 acetonitrile:0.1% formic acid for 1 hour on an orbital shaker. Method detection limits range from 0.3 to 2 ng/ml depending on media and analyte. Matrix effects of media are minimized through the use of multiple reaction monitoring versus selected ion recording. Upper confidence limits on accuracy meet the National Institute for Occupational Safety and Health 25% criterion for PTFE and PW media for all analytes. Using PTFE and PW analyzed with multiple reaction monitoring, the method quantifies levels among the different quats compounds with high precision (<10% relative standard deviation) and low bias (<11%). The method is sensitive enough with very low method detection limits to capture quats on air sampling filters with only a 15-min sample duration with a maximum assessed storage time of 103 days before sample extraction. This method will support future exposure assessment and quantitative epidemiologic studies to explore exposure–response relationships and establish levels of quats exposures associated with adverse health effects.

### Keywords

cleaning products; liquid chromatography tandem mass spectrometry; quaternary ammonium compounds; sampling and analysis methods

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### Supplementary material

Supplementary data are available at *Annals of Work Exposures and Health* online.

### Declaration

The authors declare no conflict of interest relating to the material presented in this article. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health (NIOSH). Mention of any company or product does not constitute endorsement by NIOSH. In addition, citations to Web sites external to NIOSH do not constitute NIOSH endorsement of the sponsoring organizations or their programs or products. Furthermore, NIOSH is not responsible for the content of these Web sites. Its contents, including any opinions and/or conclusions are solely those of the authors.

## Introduction

Quaternary ammonium compounds (quats) are a family of chemicals that possess antimicrobial properties. As a result, quats have found application as antiseptics, disinfectants, detergents, and preservatives in a wide range of products that span from performance textiles (Musante 2016) to cleaners and disinfectants (Loddé *et al.*, 2012; Gonzalez *et al.*, 2014). The presence of quats in consumer and commercial products raises the potential for human exposure (Dao *et al.*, 2012). Spraying, wiping, and washing surfaces such as floors and countertops or medical devices (e.g. instruments), and skin cleansing with products that contain certain quats are associated with allergic or irritant asthma and contact dermatitis among healthcare, food preparation, and cleaning workers as well as the general public (Bernstein *et al.*, 1994; Burge and Richardson 1994; Kie - wierczy ska and Kr cisz 2000; Purohit *et al.*, 2000; Nettis *et al.*, 2002; Jowsey *et al.*, 2007; Suneja and Belsito 2008; Loddé *et al.*, 2012; Gonzalez *et al.*, 2014). Specifically, exposure to benzalkonium chloride (BAC) and benzethonium chloride (BEC) quats has been associated with asthma and dermatitis (Bernstein *et al.*, 1994; Burge and Richardson 1994; Kie - wierczy ska and Kr cisz 2000; Purohit *et al.*, 2000; Nettis *et al.*, 2002; Benjamin *et al.*, 2012; Dao *et al.*, 2012).

Quats have the generic chemical structure  $N(R_1R_2R_3R_4)^+Cl^-$ , where R = alkyl group or aryl group of varying carbon chain length. BAC is a mixture of homologs with varying *n*-alkyl chain length (where *n* = number of carbons); common homologs include benzyldimethyldodecylammonium chloride (BAC12), benzyldimethyltetradecylammonium chloride (BAC14), and benzyldimethylhexadecylammonium chloride (BAC16) (Ford *et al.*, 2002; Núñez *et al.*, 2004). Basketter *et al.*, (2004) reviewed literature related to BAC-allergic properties and found BAC to be a strong skin irritant. Larsen *et al.*, (2012) reported pulmonary irritation and inflammation in mice exposed to BAC. BEC has a 27 carbon chain length alkyl group. Dao *et al.*, (2012) found BAC and BEC to be skin irritants and rare sensitizers. Some quat compounds such as (2,3-dihydroxypropyl)trimethylammonium chloride and the dialkyl ester of triethanol ammonium methyl sulfate appear to lack substantial allergic properties (Jowsey *et al.*, 2007). Dermal and respiratory irritation and sensitization potential of didecyldimethylammonium bromide (DDAB) is unknown.

Quat compounds have low vapor pressure requiring a multiple pathway approach to sampling (Saito *et al.*, 2015). Use of spray products that generate liquid aerosol that contains quats may lead to high inhalation exposures. In addition, quats may be inhaled directly or attach to solid airborne particles and be inhaled. On the other hand, their low volatility means that quats may not become airborne when applied by wiping or washing (Vincent *et al.*, 2007) but rather would persist on surfaces, potentially leading to dermal exposure. As such, a reliable analytical method is needed for an accurate assessment of inhalation and dermal exposures to quats.

In a 1993 study, Hill added known amounts of benzalkonium bromide and BAC14, BAC16, and benzyldimethyloctadecylammonium chloride quats to separate filters (type unspecified) and quantified masses using high-pressure liquid chromatography with ultraviolet detector

(HPLC-UV) (Hill 1993). Recoveries of individual quat compounds averaged about 90%; however, UV detection is not specific, can be affected by coeluting compounds, and is limited to compounds that possess chromophore groups (Ford *et al.*, 2002; Vincent *et al.*, 2007). In another study, Ford *et al.*, (2002) identified and quantified alkyl benzyl and dialkyl quats (BAC12, BAC14, BAC16; DDAB) using reversed-phase liquid chromatography with electrospray ionization quadrupole mass spectrometry detection (LC-ESI-MS) (Ford *et al.*, 2002). The method was successfully employed for the analysis of a surface wipe sample from an occupational setting. Núñez *et al.*, 2004 reported the use of LC-MS-MS for the determination of BAC homologs in spiked water samples and commercial products with high sensitivity. More recently Vincent *et al.*, (2007) reported a method specific to identification and quantification of didecyltrimethylammonium chloride (DDAC) in air. In that study, hospital air was sampled using silica gel and XAD-2<sup>®</sup> resin in tubes followed by analysis with ion chromatography. The authors noted that ion chromatography has high sensitivity and specificity for DDAC; however, levels of DDAC in hospital air were not detectable. As such, for sampling of trace amounts of quats in air, the authors recommended use of XAD-2<sup>®</sup> resin (quats formed polar bonds with silica gel) with preconcentration or use of LC-MS-MS to improve detection. In addition to increased sensitivity, LC-MS-MS allows for simultaneous differentiation, detection, and quantification of quats compounds (Ford *et al.*, 2002; Núñez *et al.*, 2004).

Despite previous work to quantify quats, a robust method is still lacking. Because of their low vapor pressure, quats are unlikely to exist in the vapor phase, rather airborne quats would likely be solid particles or dissolved in liquid droplets aerosolized from the use of spray products, thus requiring aerosol sampling techniques. Herein, we report the development of an ultraperformance LC-MS-MS (UPLC-MS-MS) method for air samples of quats (Fig. 1) collected on filter media and surface samples collected on wipe media. This new method is sensitive enough to detect quats on air sampling filters with only a 15-min sample duration with a maximum assessed storage time of 103 days before sample extraction. Method sensitivity, using sampling durations on the order of minutes, is crucial to assess exposures during short-term cleaning tasks that may generate only small amounts of quats.

## Methods

### Chemicals and materials

BEC (99%), DDAB (98%), BAC12 (puriss, 99 %), BAC14 (puriss, 99 %), BAC16 (99%), hexadecyltrimethylammonium bromide (HDTAB, 99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HDTAB was used as an internal standard (ISTD) for BEC and DDAB. Deuterated ISTDs (BAC12-d5, BAC14-d5, and BAC16-d5, 99%) were purchased from CDN Isotopes Inc. (Pointe Claire, Quebec). LCMS-grade acetonitrile, 2-propanol, formic acid, and ammonium formate were purchased from Thermo Scientific (Waltham, MA, USA). Water (18 M $\Omega$ ) was cleaned by a Millipore Milli-Q system (Billerica, MA, USA). Polypropylene centrifuge tubes (15 ml) were purchased from Fisher Scientific. All samples and standards were passed through regenerated cellulose membrane filters (0.2- $\mu$ m pore size, 15-mm i.d., Phenomenex, Torrance, CA, USA).

## Air sampling

The collection media evaluated in this study included 37-mm diameter, 1- $\mu$ m pore size glass fiber (GFF) filters, and polytetrafluoroethylene (PTFE) filters, which were found to be acceptable for air sampling. The sampling media was housed in 37-mm conductive closed-face cassettes to minimize particle adhesion to walls. Operating flow rates depend on media and sampling times due to differences in pressure drop between filter types. For sampling duration lasting 15 min or less, flow rates of 6.5 l per min (LPM) for GFF and 5.5 LPM for PTFE resulted in acceptable pressure drop (9.8 inches of water for GFF and 10 inches of water for PTFE) over the sampling duration. For sampling duration of 8 h, flow rates of 3 LPM for GFF and 1.5 LPM for PTFE resulted in acceptable pressure drop (4.2 inches of water for GFF and 4.3 inches of water for PTFE) over the sampling duration.

## Surface sampling

A preliminary study was conducted to identify a suitable material and moistening agent for surface sampling. The materials tested were alcohol prep pads (polypropylene saturated with 70% isopropyl alcohol), WypAll® X60 (laboratory towel, pulp fibers on polypropylene), gauze pads (100% woven cotton), and Pro-wipe® 880 (PW: non-woven polypropylene). All materials except for the alcohol prep pads were moistened with 300  $\mu$ l of water or 2-propanol. The experiment consisted of testing recoveries of analytes sampled from an ideal, reference surface (glass).

For surface sampling, PW cloth was cut into a square section measuring 5 cm on edge and moistened with 300  $\mu$ l of 2-propanol. The sampling area was covered by a 100-cm<sup>2</sup> template and wiped according to instructions presented in Fig. 2 (adapted from Brookhaven National Laboratory wipe sampling protocol) (Brookhaven National Laboratory 2014). If the sample area deviates from an ideal 100 cm<sup>2</sup> surface such as a door knob or computer keyboard, the area should be estimated and recorded on a sampling sheet. All laboratory experiments to assess the surface sampling technique for recovery, storage, and matrix effects (MEs) were conducted using 300  $\mu$ l of 2-propanol.

## Solution preparation

Individual analyte calibration standard (BAC12, BAC14, BAC16, BEC, and DDAB) and ISTD (BAC12-d5, BAC14-d5, BAC16-d5, and HDTAB) stock solutions of approximately 1 mg/ml were prepared by weighing 50 mg of each material in separate 50-ml volumetric flasks and diluting with LCMS grade acetonitrile. All powders and sample transfer supplies like scoops and weigh boats were passed through an antistatic loop prior to weighing.

Working ISTD solutions were prepared by adding 250  $\mu$ l of each stock ISTD solution into 25-ml volumetric flask and filling to volume with acetonitrile. The extraction solvent was prepared in two steps: first, a 60:40 acetonitrile:0.1% formic acid solution was made by adding 200-ml water and 0.5 ml of LCMS grade formic acid to a 500-ml volumetric flask and filling to the mark with acetonitrile; next, 0.49 ml of 10- $\mu$ g/ml ISTD was added to 200 ml of the solution prepared in the first step to make a 0.0245- $\mu$ g/ml concentration ISTD extraction solvent. Working calibration standard solutions were prepared by adding 250  $\mu$ l of each stock into 25-ml volumetric flask and diluting with acetonitrile to the mark. Calibration

standard points were prepared by serially diluting the working standard with the extraction solvent to make eight standard concentrations between 100 ng/ml and 0.35 ng/ml.

### Sample preparation and extraction

Sample media extraction was standardized with 60:40 acetonitrile:0.1% formic acid (extraction solvent) solution. Filter samples and filter support pads were removed from the cassette, folded inward twice, and extracted in separate vessels to assess breakthrough onto the support pad. Both filter and wipe samples were placed in the bottom of a 15-ml polypropylene tube. Samples were extracted in 5 ml of extraction solvent for 1 hour on an orbital shaker at 425 rpm and spiked with 100  $\mu$ l of 1.25  $\mu$ g/ml IS working standard prior to analysis. For filter samples, the cassette walls were rinsed with 1 ml of 60:40 acetonitrile:0.1% formic acid solution (extraction solvent); 0.5 ml of the rinse was combined with 0.1 ml of ISTD at 150 ng/ml and analysed.

### Analytical

**Instrumentation**—A calibrated electronic microbalance (Model UMX2, Mettler Toledo, Greifensee, Switzerland) capable of reading from 0.001 to 10 mg (i.e. six-place balance) was used to weigh quat standards. A UPLC system (Acquity FTN, Waters, Milford, MA, USA) attached to a XEVO TQD (Waters) with an ESI interface was used for the determination of quats. The mass spectrometer was operated in positive ion mode under the following conditions: capillary voltage 3.00 kV, cone voltage 44 V, RF 2.5 V, extractor 3V, source temperature 150°C, desolvation temperature 500°C, desolvation gas flow 900 l/h, and cone gas flow 20 l/h. MassLynx software (Waters) was used to control the UPLC-MS-MS system, and TargetLynx (Waters) was used to process the data.

**Analytical procedure**—The analytes were separated on an Acquity UPLC<sup>®</sup> HSS Cyano 1.8  $\mu$ m column (2.1  $\times$  100 mm, Waters) maintained at 40°C. A cyano column was chosen pursuant to Ford *et al.*, 2002. The cyano functional group of the column provided varying interaction with the different alkyl chain lengths of the BAC compounds ensuring effective chromatographic separation (Fig. 3). A traditional reversed-phase C18 column was also assessed but did not separate the BACs. An isocratic mobile phase program, 60:40 acetonitrile:100-mM ammonium formate/0.1% formic acid (pH 3.5) flowing at 0.4 ml/min, was used to separate the quats. The injection volume was 10  $\mu$ l. The acquisition program was set to 1.5 min after the last eluting compound (total run time 5.5 min) to allow contaminants to pass through the column prior to the next sample injection. The mass spectrometry system was operated in multiple reaction monitoring (MRM) and selected ion recording (SIR) modes.

MS-MS parameters such as quantifier and qualifier transitions and ions, retention time, and cone voltages are provided in Table 1. Mass acquisition span was set to 0.3 Daltons to accommodate for small mass shifts away from the target ion mass. The daughter ions for quantifiers and qualifiers of BAC12, BAC14, and BAC16 are due to the breaking of the carbon–nitrogen bond between the quaternary amine and the benzyl substituent (Takeoka *et al.*, 2005). Loss of toluene ( $m/z$  92 mass difference) from the BAC homologs creates the

most stable and abundant response for quantitation. Loss of the tertiary amine from the intact cation leaves a benzyl ion.

### Method assessment

**Calibration**—Calibration stock solutions were made monthly in acetonitrile and stored at 4°C. Working and calibration standards in the extraction solvent were made as needed. Calibration ranged from the detection limit of 0.35 ng/ml to 100 ng/ml. Calibration vials were only used once to limit losses due to septa piercing. Linear regressions of relative response of ISTD to the analyte weighted by the inverse of the concentration were used for calibrations to address possible nonlinear relationship due to analytes spanning multiple orders of magnitude of concentrations or heteroscedasticity in the data.

**Method detection limits**—Method detection limits (MDLs) were assessed by spiking each media with eight low-level standards in acetonitrile from below the estimated LOD to 10 times the LOD (0.05–5.0 ng/ml). To mimic field samples, surface media (PW) were spiked in centrifuge tubes after being moistened with 2-propanol while air samples were spiked on aluminum foil trays and allowed to dry. Response was regressed against known concentrations and the root mean square error (RMSE) and slope were used to calculate MDLs as  $3 \times \text{RMSE/slope}$  or the lowest calibration standard, whichever was higher (Kennedy *et al.*, 1995).

**Bias, precision and accuracy**—To determine method accuracy, bias, and precision, media were spiked with standards in the same fashion as the detection limit study but at four concentration levels (4, 20, 40, 80 ng/ml) with nine replicates per concentration ( $n=36$  per media/analyte/method combination). Method accuracy is a combination of bias and precision of the method used by National Institute for Occupational Safety and Health (NIOSH) to determine whether a method has acceptable performance. Bias was assessed by taking the ratio of measured to theoretical value at each concentration and averaged across all concentrations (4–80 ng/ml) after testing for homogeneity of the bias using Tukey's multiple comparison test in JMP 11 (SAS Institute Inc., Cary, NC, USA) at  $\alpha=0.05$ .

$$\%bias = \left( \frac{\text{measured}}{\text{theoretical}} - 1 \right) * 100\% \quad (1)$$

Precision was calculated as the pooled relative standard deviation (RSD) across the four concentrations after testing for homogeneity of the RSDs using Bartlett's test in JMP and calculated as:

$$\text{RSD}_k = \frac{\sqrt{\sum_1^i (x_i - \bar{x})^2 / N_k}}{\bar{x}} \quad (2)$$

$$RSD = \sqrt{\sum_{k=1}^4 \frac{(N_k - 1)(RSD_k^2)}{k \times (N_k - 1)}} \times 100 \quad (3)$$

where  $RSD_k$  is the RSD at the  $k$ th concentration,  $x_i$  is the  $i$ th measurement (generally nine measurements per concentration) at the  $k$ th concentration,  $\bar{x}$  is mean of  $k$ th concentration,  $N_k$  is number of samples in  $k$ th concentration, and  $k$  is four concentrations.

Accuracy was calculated as:

$$A = \begin{cases} u_{(1+\alpha)/2} \times [\text{bias}^2 + RSD^2]^{\frac{1}{2}}, & |\text{bias}| < \frac{RSD}{u_{\alpha}} \\ |\text{bias}| + u_{\alpha} \times RSD, & \text{otherwise} \end{cases} \quad (4)$$

where  $u_{\alpha}$  is a unit normal quantile equal to 1.645 at a 95% confidence level (Bartley 2001). The 95% upper and lower confidence limits on accuracy were calculated according to Bartley 2001 and compared to the NIOSH 25% accuracy criterion.

**Storage stability**—Analyte storage stability was assessed at 40 ng/ml concentration for 103 days on each media with six replicates per day under two conditions: refrigerated (4°C) and room temperature (22°C). The first measurement was made on Day 0, followed by measurements on Days 10, 22, 34, and 103. Day 0 values were used to calculate bias. A change of greater than  $\pm 10\%$  bias was deemed unacceptable.

**Matrix effects**—Media matrix can affect method performance in terms of ion selectivity and reliability by either enhancing or suppressing ions during identification and quantification using LC-MS-MS systems. Effects were tested using six replicates at seven concentrations: 1, 5, 10, 25, 50, 75, and 100 ng/ml. MEs, recovery effects (REs), and process efficiency (PE) were assessed by spiking analyte and ISTD before and after extraction and comparing instrument area response to that from neat analytical standards at the same concentration level (Matuszewski *et al.*, 2003) as:

$$ME(\%) = \frac{\text{Area}_{\text{after}}}{\text{Area}_{\text{neat}}} \times 100 \quad (5)$$

$$RE(\%) = \frac{\text{Area}_{\text{before}}}{\text{Area}_{\text{after}}} \times 100 \quad (6)$$

$$PE(\%) = \frac{\text{Area}_{\text{before}}}{\text{Area}_{\text{neat}}} 100 \quad (7)$$



MEs in terms of ion suppression or enhancement for each analyte at each concentration were evaluated by comparing analyte area spiked after extraction to that of neat standards so changes in response do not include those from the extraction. RE includes changes in response due to the extraction (i.e. sample preparation portion of the process). PE includes all changes in response from both the matrix and sample preparation and is a combination of ME and RE.

**Surface recoveries**—Surfaces spiked with 100 µl of a 1-mg/ml stock in acetonitrile were allowed to dry and then sampled with PW moistened with 2-propanol according to the wiping procedure described earlier. Alcohol loss was determined gravimetrically and accounted for in the recovery calculation. Types of surfaces spiked and wiped included: glass plates, stainless steel (metal), and epoxy-coated laboratory bench top (epoxy). Recoveries of greater than 50% using a single wipe sample were deemed acceptable.

**Field sampling**—A pilot study was conducted to collect air and surface samples from a hospital where cleaning and disinfecting products were used. The air sampling consisted of PTFE filters collected as described earlier. The wipe samples were collected using PW media. No correction was made for residual alcohol on the PW media as this will vary by surface. Laboratory estimates indicate a maximum of 1.8% error in extraction volume (glass, metal, and epoxy surfaces tested).

## Results

### Calibration

The calibration curves were generated using the standard relative response of the ITSD and analyte signals for each analyte and method (MRM or SIR); these were deemed linear upon visual inspection (Supplementary Table S1). All calibration curves showed a good linear fit ( $R^2 > 0.997$ ). Calibration curves were used to calculate the recoveries for the determination of LOD, accuracy, MEs, and stability studies.

### Method detection limits

Calculated MDLs for sampling media ranged from 0.3 to 2 ng/ml for the different quats compounds using MRM and 0.1 to 2.0 ng/ml using SIR (Supplementary Table S2). GFF generally had the lowest MDLs of all media types (0.3–0.6 ng/ml) except for BEC on GFF. MDLs were calculated as  $3 \times \text{RMSE/slope}$  for all media and quats compounds except for DDAB on PTFE and PW using MRM, which were set to the lowest measured concentration used in the assessment.

### Bias, precision, and accuracy

Bias and precision were calculated for each combination of media, analyte, and method (Fig. 4). When bias was heterogeneous across concentration levels (asterisks above hashed bars in Fig. 4), maximum bias was used for calculating accuracy. Bias generally fell within  $\pm 10\%$  (dotted line), which is the NIOSH criterion for acceptable bias, except for the following: BEC on PW using MRM method; BEC on PTFE using SIR method; and BEC and DDAB on PW using SIR method. When precision was heterogeneous across levels (asterisks above



solid black bars in Fig. 4), maximum RSD from the four nominal concentration levels was used for calculating accuracy. The calculated RSDs, or precision estimates, were all less than 10%, except for BAC16 and BEC on GFF using MRM.

Upper (95%) and lower (5%) confidence limits on accuracy were calculated for each media, analyte, and method combination (Fig. 5). Upper confidence limits on accuracy were below 25% except for the following: BAC14, BAC16, and BEC on GFF using MRM; BEC on PTFE using SIR; and BEC and DDAB on PW using SIR. When the confidence limits encompassed 25%, the experimental result was inconclusive in terms of the NIOSH accuracy criterion. When the lower confidence limit was above 25% (i.e. bottom of the floating bar above 25%), the condition failed the NIOSH accuracy criterion. These conditions could be improved by applying a bias correction. Nevertheless, PTFE and PW using MRM showed acceptable accuracy. PTFE filters are recommended for sampling airborne quats or quat-containing particulate. Overall, SIR was the best performer for the accuracy experiments but is influenced by MEs (reported in the next section). MRM is the most appropriate analytical method in terms of a combination of accuracy and minimization of matrix interferences.

### Storage stability

All analytes were stable for 103 days at refrigerated and room temperature with bias of less than 10% compared to Day 0 measurements on all media types using MRM (Fig. 6 top). Since SIR Day 0, measurements showed up to 18% negative bias (Supplementary Fig. S1), SIR storage data were compared to theoretical values (Fig. 6 bottom). BEC was not stable on PW using SIR. Quats on GFF and PTFE were stable for 103 days at both temperature conditions using SIR.

### Matrix effects

Mean percentage RE, regardless of method (MRM or SIR) or compound, for GFF was approximately 100% recovery while PTFE was slightly lower, around 90% recovery (Fig. 7, top). PW mean recoveries were approximately 95%. A number of outliers were identified by visual inspection of box plots but retained in the analysis (data not shown) as removal did not influence the overall interpretation of the data in aggregate. Percentage ME mean and 95% confidence limits show increased variability with SIR versus MRM and a slight matrix enhancement of SIR method as evidenced by mean percentage MEs above 100% (Fig. 7, middle). Percentage PE showed smaller confidence intervals for MRM over SIR (Fig. 7, bottom). Most analytes on GFF and PW had mean process efficiencies of approximately 100% while all analytes on PTFE had mean process efficiencies of approximately 92% (Fig. 7, bottom).

### Surface recoveries

WypAll® X60 moistened with water had recoveries between 49% and 56%. Gauze moistened with water and alcohol prep pads had recoveries between 76% and 83% depending on analyte. PW moistened with water had recoveries around 60%. PW moistened with 2-propanol was the best at recovering quats from a surface. Recoveries of all three BAC analytes were greater than 90% when sampling from an ideal, glass surface after a single

wipe (Supplementary Table S3). Recoveries were the worst from an epoxy resin-coated laboratory bench followed by a stainless steel metal cart. Nevertheless, recoveries of all analytes from all surfaces except for BEC on epoxy resin surface were deemed acceptable (>50%) according to OSHA guidelines for surface sampling (OSHA 2016). BAC compounds are slightly more polar than BEC and DDAB. BEC and DDAB recoveries were generally worse than the BAC compounds presumably due to differences in polarity and solvent-analyte-surface interactions since 2-propanol is slightly polar (compared to water).

### Field sampling

All field samples were measured using MRM method with filter samples separated into filter, support pad, and cassette rinse. For PTFE, a majority of the sample was collected on the filter (median 69.7%–79.3% for BAC12 to BAC16) with generally smaller fractions on cassette rinse (9.53%–23.7%) and support pad (4.2%–6.6%). For GFF, only BAC14 at 85.7% was quantified on the filter with BAC12 and BAC16 found mostly on the cassette rinse. For some samples, nothing was collected on the filter, but measurable masses were collected on the cassette and the support pad. Including a separate analysis of the three sampler components or a combination of the components into a single sample metric is important to accurately represent the airborne concentration of quats. A short-duration (15 min) area air sample measured 0.23  $\mu\text{g}/\text{m}^3$  BAC12, 1.5  $\mu\text{g}/\text{m}^3$  BAC14, and 0.96  $\mu\text{g}/\text{m}^3$  BAC16 while a housekeeper actively cleaned a hospital bathroom with a Virex TB<sup>®</sup> spray containing BAC at less than 1% by weight. BAC proportions in the air sample (56% BAC14, 36% BAC12, and 8.6% BAC16) are comparable to that in solution (60% BAC14, 30% BAC16, and 5% BAC12) based on the Safety Data Sheet. The chromatogram for this sample is displayed in Fig. 3. A short-duration (15 min) area air sample measured 3.5  $\mu\text{g}/\text{m}^3$  of BAC12, 1.2  $\mu\text{g}/\text{m}^3$  of BAC14, 0.15  $\mu\text{g}/\text{m}^3$  of BAC16, and 0.46  $\mu\text{g}/\text{m}^3$  of BEC while housekeeper swept a hospital waiting room; no quats products were used during this task. This demonstrated that the method was capable of collecting quats in the air over short periods from nonspraying sources. Full-shift (8 h) area air samples detected 0.011  $\mu\text{g}/\text{m}^3$  of BAC12, 0.028  $\mu\text{g}/\text{m}^3$  of BAC14, and 0.006  $\mu\text{g}/\text{m}^3$  of BAC16 in locations where quat-containing cleaning and disinfecting products were generally used. Surface samples of a nurse's station revealed 6.9 to 21.8  $\mu\text{g}/100\text{ cm}^2$  BAC12, 23.8 to 76.6  $\mu\text{g}/100\text{ cm}^2$  BAC14, and 10.5 to 28.8  $\mu\text{g}/100\text{ cm}^2$  BAC16.

### Discussion

A method is presented here for the collection, analysis, and quantification of multiple quats compounds in air and on surfaces. This UPLC-MS-MS method to quantify the different quats compounds is superior to existing methods which use HPLC-UV (Hill 1993), LC-ESI-MS (Ford *et al.*, 2002), ion chromatography (Vincent *et al.*, 2007), and LC-MS-MS (Núñez *et al.*, 2004) in terms of sampling technique as well as separation, identification, and accuracy. The method reported here is very sensitive with MDLs ranging from 0.3 to 2 ng/ml for the different quats compounds on different media using MRM method, compared to existing analytical methods whose LODs ranged from 0.1 to 560 ng/ml (Hill 1993; Ford *et al.*, 2002; Núñez *et al.*, 2004; Vincent *et al.*, 2007). These differences in sensitivity were due to instrumentation and the method for calculating a detection limit.

The method reported here achieved acceptable biases mostly below 10% depending on compound, media and method, high precision with RSDs mostly below 10%, and good accuracy with the upper 95% confidence limit less than 25% for most combinations of compound, media, and method. The method showed very few MEs for MRM method, but some ion enhancement was observed for the SIR method, which could be dealt with by using a matrix-matched bias correction. SIR method may be a viable alternative to MRM when only single quadrupole instrumentation is available. In the tests for storage stability, samples were shown to be stable for over 3 months before extraction. Finally, the pilot air sampling results demonstrate that airborne quat compounds can be collected on filter media and quantified at very low concentrations and over very short-sampling durations. Rinsing the cassette and including the support pad in the analysis is warranted based on separate component analysis presented here. Improvements to the air sampling component of this technique would be the assessment of the aerosol sampler effect on collection of quats from dynamically generated test atmospheres in a controlled chamber study.

The analysis of the sampler components (i.e. wall, support pads, stage components) could be investigated using these chamber tests. We used 37-mm cassette samplers for proof-of-concept; however, validation of the collection media enables the use of this analytical technique in conjunction with alternative samplers such as those designed to collect the inhalable (e.g. IOM sampler) or respirable (e.g. cyclones) aerosol fractions. Previous studies of air monitoring for quats compounds using vapor sampling methods did not yield quantifiable levels (Vincent *et al.*, 2007). Surface sampling also demonstrated that quats can be quantified using the surface wipe sampling method with reasonable reliability based on recoveries of 45%–96% from different surfaces. Successive wipes from the same surface could increase recoveries when necessary.

To date, a major barrier to better understanding exposure–response relationships for quats has been the absence of a robust analytical method that can be used for multiple pathway (surface and air) exposure assessment approaches (Saito *et al.*, 2015). This method can detect very low levels of quats in the air (present as quats particles or aerosolized product droplets) and a variety of surfaces commonly encountered in healthcare, veterinarian, food preparation, and other industrial or household surfaces and hence provides an invaluable tool for quantification of quats in different environments. The development of this method enables quantitative epidemiologic studies to explore exposure–response relationships and quantify levels of quats exposure associated with adverse effects.

## Conclusions

A new method was designed for sampling select quats in air or on surfaces and analysis by UPLC tandem mass spectrometry. The method accuracy meets the NIOSH accuracy criterion and can quantify levels among the different quat compounds with high precision and low bias. The method is sensitive enough with very low LODs to capture quats on air sampling filters with only a 15-min sample duration with a maximum assessed storage time of 103 days before sample extraction. Based on accuracy and MEs, the recommended sampling media are PTFE for air sampling and PW for surface sampling. This method will

support future exposure assessment and epidemiologic studies to explore exposure–response relationships and establish levels of quats exposure associated with adverse effects.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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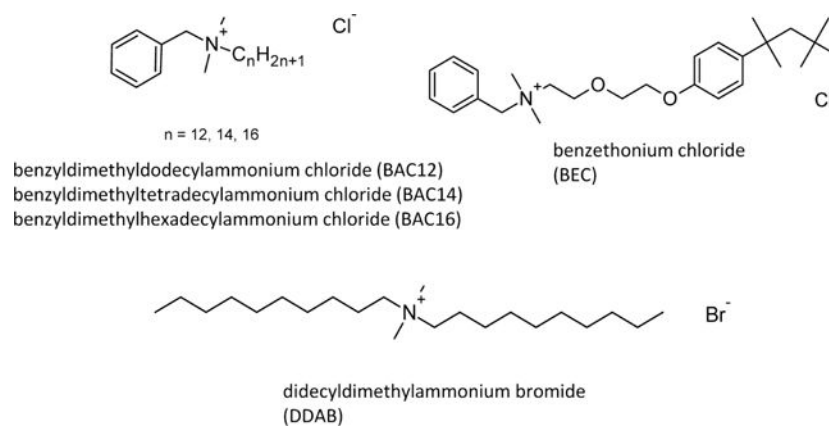
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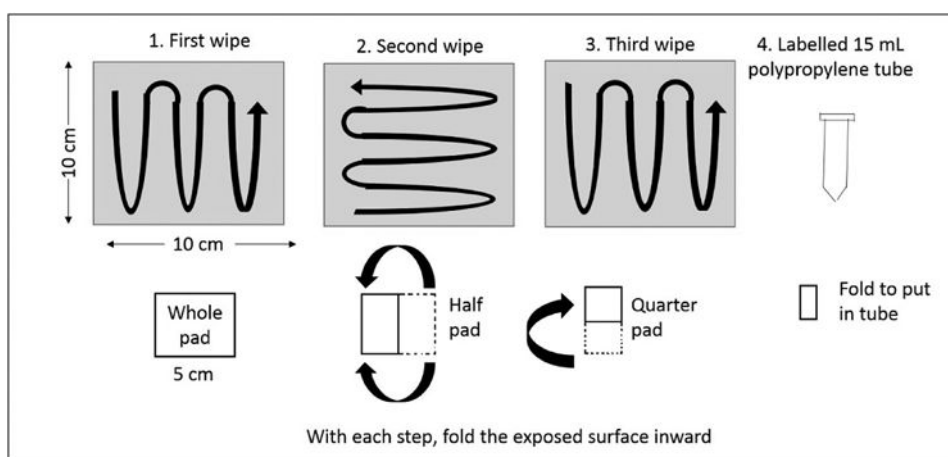
## References

- Bartley DL. Definition and assessment of sampling and analytical accuracy. *Ann Occup Hyg.* 2001; 45:357–64. [PubMed: 11418085]
- Basketter DA, Marriott M, Gilmour NJ, et al. Strong irritants masquerading as skin allergens: the case of benzalkonium chloride. *Contact Dermatitis.* 2004; 50:213–7. [PubMed: 15186375]
- Benjamin B, Chris F, Salvador G, et al. Visual and confocal microscopic interpretation of patch tests to benzethonium chloride and benzalkonium chloride. *Skin Res Technol.* 2012; 18:272–7. [PubMed: 22142417]
- Bernstein JA, Stauder T, Bernstein DI, et al. A combined respiratory and cutaneous hypersensitivity syndrome induced by work exposure to quaternary amines. *J Allergy Clin Immunol.* 1994; 94(2 Pt 1):257–9. [PubMed: 8064078]
- Brookhaven National Laboratory. IH75190: Surface wipe sampling procedure. 2014. Available at [https://www.bnl.gov/esh/shsd/sop/pdf/ih\\_sops/ih75190.pdf](https://www.bnl.gov/esh/shsd/sop/pdf/ih_sops/ih75190.pdf). Accessed October 20, 2016
- Burge PS, Richardson MN. Occupational asthma due to indirect exposure to lauryl dimethyl benzyl ammonium chloride used in a floor cleaner. *Thorax.* 1994; 49:842–3. [PubMed: 8091336]
- Dao H Jr, Fricker C, Nedorost ST. Sensitization prevalence for benzalkonium chloride and benzethonium chloride. *Dermatitis.* 2012; 23:162–6. [PubMed: 22828255]
- Ford MJ, Tetler LW, White J, et al. Determination of alkyl benzyl and dialkyl dimethyl quaternary ammonium biocides in occupational hygiene and environmental media by liquid chromatography with electrospray ionisation mass spectrometry and tandem mass spectrometry. *J Chromatogr A.* 2002; 952:165–72. [PubMed: 12064528]
- Gonzalez M, Jégu J, Kopferschmitt MC, et al. Asthma among workers in healthcare settings: role of disinfection with quaternary ammonium compounds. *Clin Exp Allergy.* 2014; 44:393–406. [PubMed: 24128009]
- Hill, G. Analytical report: Benzalkonium bromide and chloride. Cincinnati, OH: Datachem Laboratories; 1993.
- Jowsey IR, Kligman AM, White IR, et al. Evidence that two alkyl ester quaternary ammonium compounds lack substantial human skin-sensitizing potential. *Dermatitis.* 2007; 18:32–9. [PubMed: 17303042]
- Kennedy, ER., Fischbach, TJ., Song, R., Eller, PM., Shulman, SA. Guidelines for air sampling and analytical method development and evaluation. Cincinnati, OH: National Institute for Occupational Safety and Health; 1995. p. 95-117.
- Kie - wierzyska M, Krzyszk B. Occupational skin diseases among the nurses in the region of Lodz. *Int J Occup Med Environ Health.* 2000; 13:179–84. [PubMed: 11109741]
- Larsen ST, Verder H, Nielsen GD. Airway effects of inhaled quaternary ammonium compounds in mice. *Basic Clin Pharmacol Toxicol.* 2012; 110:537–43. [PubMed: 22188809]

- Loddé B, Paul M, Roguedas-Contios AM, et al. Occupational dermatitis in workers exposed to detergents, disinfectants, and antiseptics. *Skinmed*. 2012; 10:144–50. [PubMed: 22783559]
- Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem*. 2003; 75:3019–30. [PubMed: 12964746]
- Musante G. Performance wear: Running to antimicrobials. *AATCC Review*. 2016; 16:30–35.
- Nettis E, Colanardi MC, Soccio AL, et al. Occupational irritant and allergic contact dermatitis among healthcare workers. *Contact Dermatitis*. 2002; 46:101–7. [PubMed: 11918604]
- Núñez O, Moyano E, Galceran MT. Determination of quaternary ammonium biocides by liquid chromatography-mass spectrometry. *J Chromatogr A*. 2004; 1058:89–95. [PubMed: 15595655]
- OSHA. Evaluation guidelines for surface sampling methods. 2016. Available at <https://www.osha.gov/dts/sltc/methods/surfacesampling/surfacesampling.pdf>. Accessed October 20, 2016
- Purohit A, Kopferschmitt-Kubler MC, Moreau C, et al. Quaternary ammonium compounds and occupational asthma. *Int Arch Occup Environ Health*. 2000; 73:423–7. [PubMed: 11007347]
- Saito R, Virji MA, Henneberger PK, et al. Characterization of cleaning and disinfecting tasks and product use among hospital occupations. *Am J Ind Med*. 2015; 58:101–11. [PubMed: 25351791]
- Suneja T, Belsito DV. Occupational dermatoses in health care workers evaluated for suspected allergic contact dermatitis. *Contact Dermatitis*. 2008; 58:285–90. [PubMed: 18416759]
- Takeoka GR, Dao LT, Wong RY, et al. Identification of benzalkonium chloride in commercial grapefruit seed extracts. *J Agric Food Chem*. 2005; 53:7630–6. [PubMed: 16159196]
- Vincent G, Kopferschmitt-Kubler MC, Mirabel P, et al. Sampling and analysis of quaternary ammonium compounds (QACs) traces in indoor atmosphere. *Environ Monit Assess*. 2007; 133:25–30. [PubMed: 17180409]

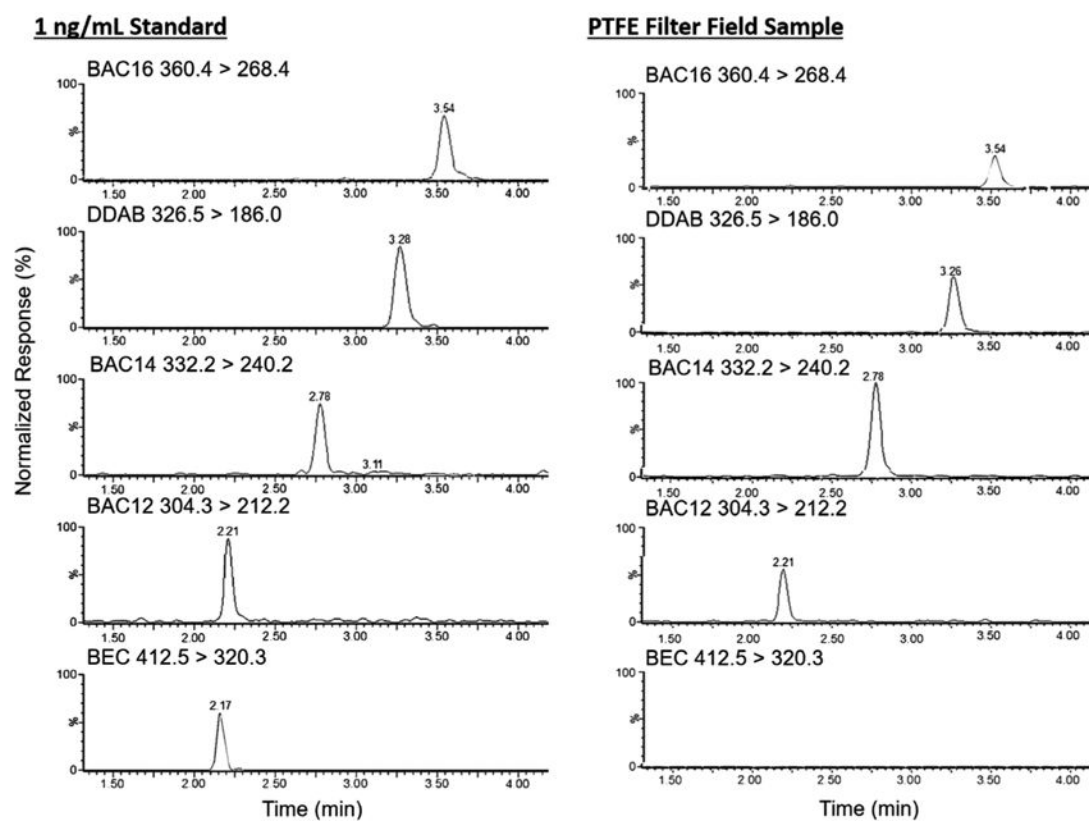


**Figure 1.**  
Chemical structures of target quaternary ammonium compounds.



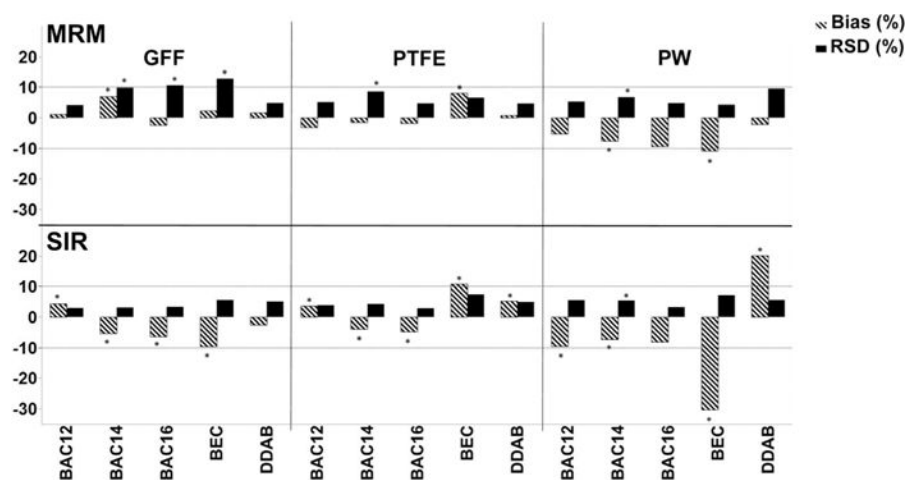
**Figure 2.**  
Surface sampling wipe method.



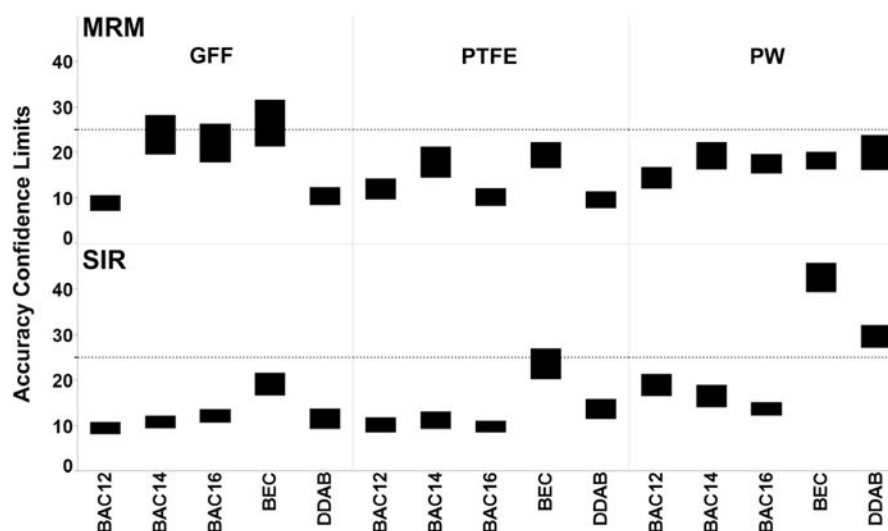


**Figure 3.**

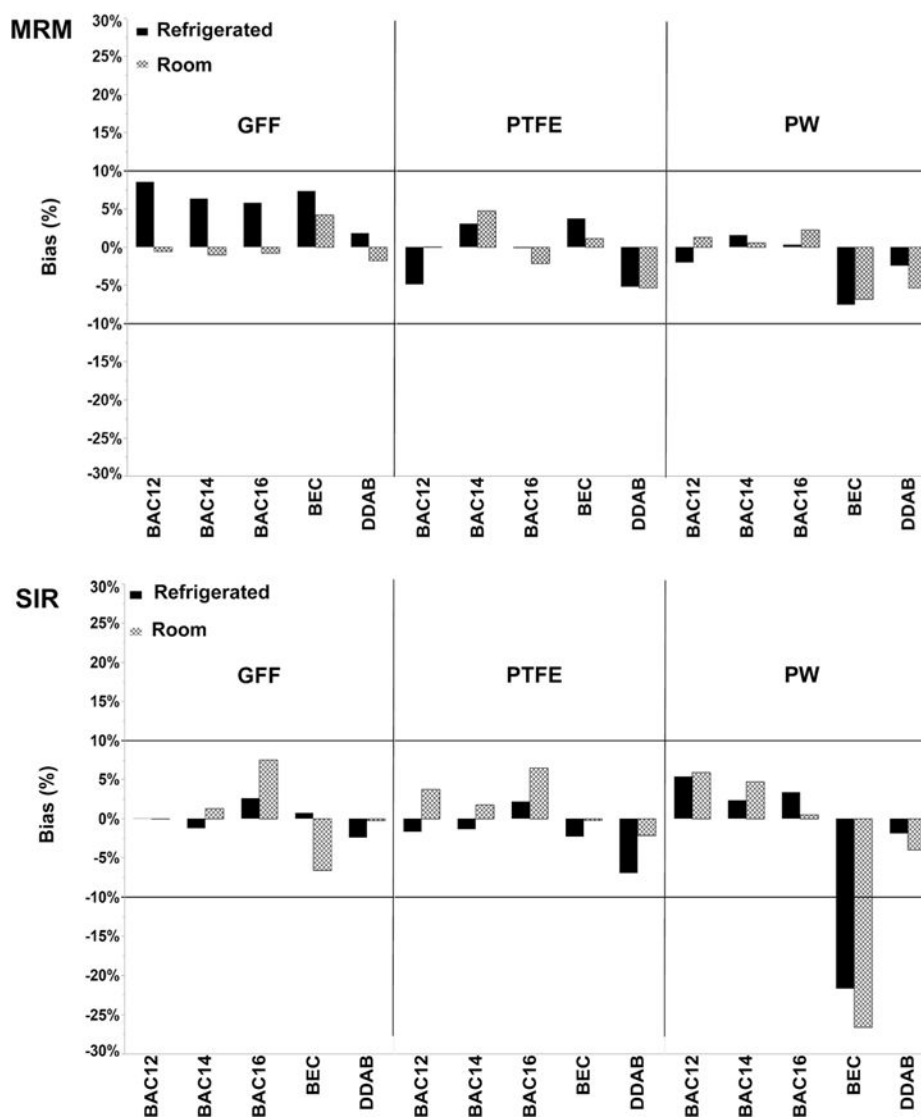
Chromatograms showing clear separation of quaternary ammonium compounds in time and/or multiple reaction monitoring transitions. Standard at 1 ng/ml (left) and polytetrafluoroethylene (PTFE) filter field sample (right).



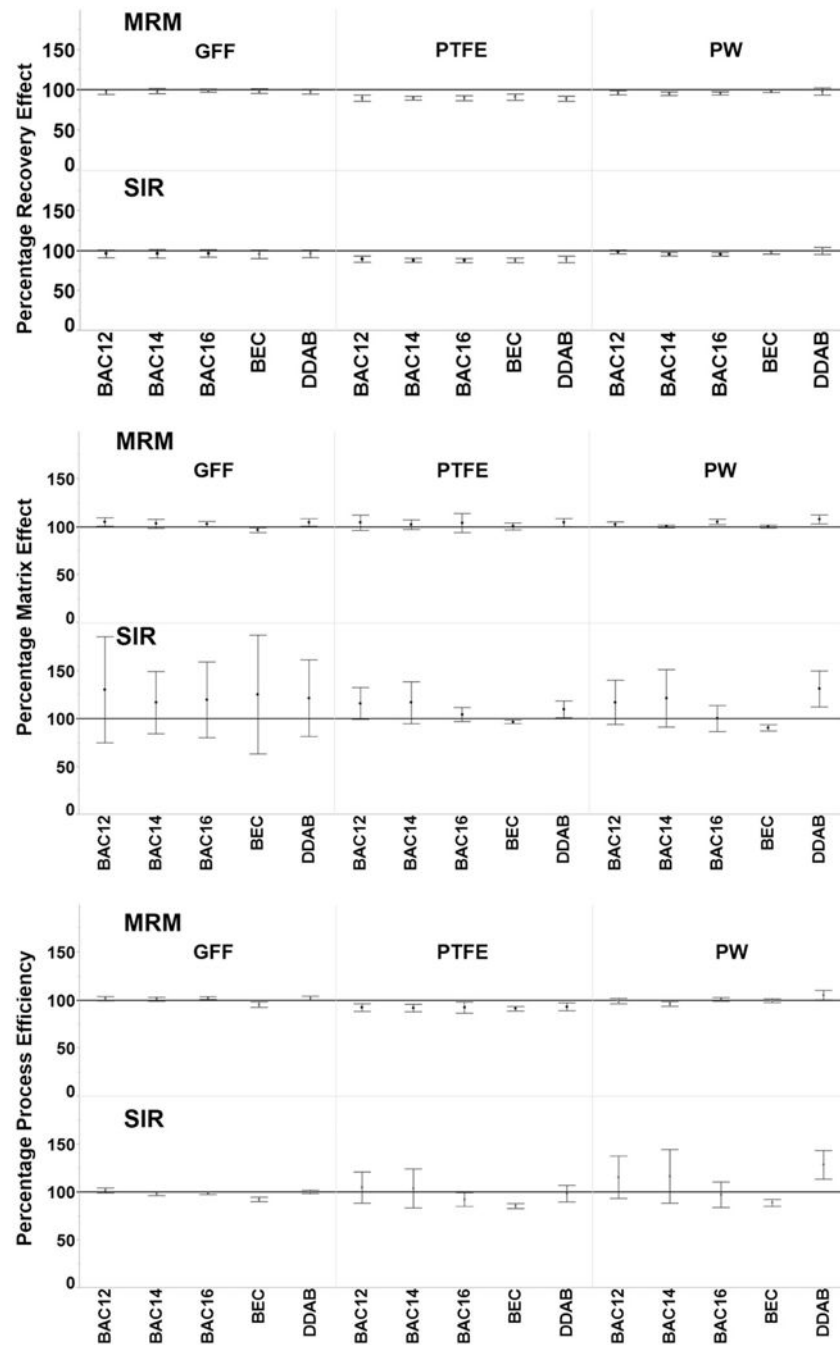
**Figure 4.** Bias and precision by media and analyte using multiple reaction monitoring (MRM top) and selected ion recording (SIR bottom).



**Figure 5.** Upper and lower confidence limit on accuracy by media and analyte using multiple reaction monitoring (MRM top) and selected ion recording (SIR bottom).

**Figure 6.**

Storage stability of quaternary ammonium compounds (% bias) by media and analyte using multiple reaction monitoring (MRM top) and selected ion recording (SIR bottom): Bias of Day 103 to Day 0.



**Figure 7.**

Percentage recovery effect (top), percentage matrix effect (middle), and percentage process efficiency (bottom) means and 95% confidence intervals.

**Table 1**

Mass spectrometer parameters for multiple reaction monitoring (MRM) and selected ion recording (SIR) methods.

Method	Compound	Quantifier transition or ion	Qualifier transition or ion	Retention time (minutes)	Cone (V)	Collision energy (Quant, Qual)
MRM	BEC	412.5>320.3	412.5>91.0	2.32	66	28, 44
	BAC12	304.3>212.2	304.3>90.9	2.37	48	20, 28
	BAC12-d5	309.5>212.3	ND	2.37	54	22
	BAC14	332.2>240.2	332.2>91.0	3.05	52	22, 36
	BAC14-d5	337.4>240.3	ND	3.05	62	24
	DDAB	326.5>186.0	326.5>85.0	3.66	74	30, 32
	BAC16	360.4>268.4	360.4>91.0	3.99	54	26, 30
	BAC16-d5	365.5>268.4	ND	3.99	64	24
	BEC	412.5	ND	2.32	66	NA
	BAC12	304.3	ND	2.37	48	NA
SIR	BAC14	332.2	ND	3.05	48	NA
	DDAB	326.5	ND	3.66	74	NA
	BAC16	360.4	ND	3.99	20	NA

ND, not determined; NA, not applicable